FURTHER STUDIES ON THE CHROMOSOMES OF THE HEMIPTERA HETEROPTERA.

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The present account deals with the relations of the chromosomes in the spermatogenesis of certain *Hemiptera*, and is practically a continuation of a previous paper of mine.[1] In the present paper will be found a description of the chromosomal relations, as far as they could be determined, in certain species not heretofore examined.

The material was collected last summer at Woods Holl, Mass., and was kindly identified for me by Dr. Philip R. Uhler, of Baltimore. The testes of some of the species (*Tingis clavata*, *Corixa verticalis*, *Cymus luridus*, *Lygus pratensis*) were fixed simply in Conklin's picro-acetic mixture, and this fixation not allowing successful staining with Hermann's saffranine-gentian violet method the relations of the nucleoli and of the chromatin nucleoli could not be determined. But in the remaining species (*Nabis annulatus*, *Corizus alternatus* and *Harmostes reflexulus*) the testes were fixed in Hermann's platinic chloride-osmic-acetic mixture which allowed the saffranine-violet stain, so that for these species the distinction of nucleoli and chromatin nucleoli could be made.

As in my preceding paper (*l. c.*) on this subject, the term "chromatin nucleolus" is applied to that peculiar nuclear element which is a chromosome peculiarly modified in preserving its form and dense structure, which chromosomes as a rule show only in the height of mitosis, throughout every stage of the spermatogenetic cycle. With the saffranine-violet method, the chromosomes proper stain red only in mitosis, and violet in other stages, but the chromatin nucleolus maintains the red stain in all stages.

1. Tingis clavata Stål.

Three testes were studied, none of them showing spermatogonic monaster stages.

[1] "A Study of the Chromosomes of the Germ Cells of Metazoa," to be published in the *Transactions of the American Philosophical Society*.

Pole views of the monaster stage of the first maturation mitosis (Pl. X, fig. 1) show seven chromatin elements of relatively equal volume, and lateral views of the chromosomes in this stage show that they are all dumbbell-shaped, and hence probably bivalent (fig. 2). This mitosis results in a transverse (reduction) division of all these elements. Very frequently one of them is seen to be characterized in having its two components of very unequal volume.

Since these preparations were stained merely by the iron-hæmotoxylin method, the presence of chromatin nucleoli could not be positively determined owing to the lack of differential staining. But in the prophases of the first maturation mitosis can be seen a large true nucleolus, and two smaller rounded bodies (generally of different volumes) which are sometimes in mutual apposition, and sometimes not. If the latter are chromatin nucleoli, they are much smaller than any of the seven bivalent chromatin elements of the maturation division, so that the latter are possibly all unmodified chromosomes.

This species of the *Tingitidæ*, in having such a small number of chromosomes, may be regarded rather as a specialized than a primitive form.

2. Corixa verticalis Fieber.

The chromosomes could not be counted in the monaster stages of the spermatogonia. Two testes were examined.

Pole views of the monaster stage of the first maturation mitosis (Pl. X, fig. 3, in which two of the chromosomes are seen laterally) show twelve chromatin elements, of which one regularly is placed in the centre of a circle composed of the remaining eleven. Lateral views (fig. 4, in which four of the large and two of the small elements are shown) show that all these elements are dumbbell-shaped, and hence probably bivalent. Three are much smaller than the remaining nine, and the very smallest is the one that occupies the centre of the chromosomal plate. All these elements divide by a transverse (reduction) division, and in the daughter cells (second spermatocytes) the chromosomes are arranged all close together in the equatorial plane; it is the case in a number of species of the *Hemiptera* that the chromosomes show different plans of arrangement in the two maturation mitoses.

In the post-synapsis stage there is found in the nucleus a peri-

pheral, compact, densely staining body of dumbbell form (Pl. X, fig. 5, the chromatin reticulum not shown in this figure). This possibly represents a true nucleolus and a chromatin nucleolus in apposition, but this point could not be determined. Similarly I could not ascertain whether the three small chromatin elements of the maturation mitoses are chromatin nucleoli.

3. Cymus luridus Stål.

There were no spermatogonic mitoses in the two testes examined of this Lygæid.

Pole views of the monaster stage of the first maturation mitosis show fifteen chromatin elements of very varying volumes (fig. 6), though one (*N. 2?*) is always much smaller than the others and, by analogy with many other *Hemiptera*, probably represents a chromatin nucleolus. Lateral views of the same stage show that all these elements are dumbbell-shaped, and so probably bivalent (fig. 7 showing the smallest and four of the larger elements). All these elements become transversely divided.

In the growth period of the spermatocytes, preceding the maturation divisions, the nucleus contains a large true nucleolus, very irregular in form and peripheral in position. There are also found as many as four smaller, rounded bodies, two of which are frequently mutually apposed; if these be chromatin nucleoli there would be potentially two bivalent chromatin nucleoli in the resting spermatocyte (four univalent ones), though apparently only a single bivalent one in the maturation mitosis.

This species has a larger number of chromatin elements in the first maturation mitosis than does the closely similar *C. angustatus*, which I have shown to possess only thirteen.

4. Lygus pratensis Linn.

The individuals of this species of Capsid were labeled by Dr. Uhler, "*Lygus pratensis* var.;" whether Dr. Uhler regarded them as simply showing slight differences in color, or as a good geographical variety, I cannot say. In the two testes studied there were no spermatogonic monasters.

In the monaster stage of the first maturation division are found eighteen chromatin elements (Pl. X, figs. 8, 9), namely, sixteen larger and two (*N. 2*) much smaller; while in the monaster of the second maturation mitosis (fig. 10) are present seventeen elements,

sixteen larger and one smaller (*N. 2*). The sixteen larger elements in the first mitosis are all bivalent, and probably are all true chromosomes; they all divide transversely. The two small elements (those marked *N. 2* in the figures) of this mitosis do not divide, but one of them goes undivided into the one daughter cell (second spermatocyte), the other one into the other—this explaining why in the first spermatocytes there are eighteen elements, in the second only seventeen. On account of these small elements not dividing, each of them must be considered univalent; for so far as my observations on the *Hemiptera* have gone, all bivalent elements divide transversely in the first maturation mitosis.

The species of *Capsidæ* thus far examined (compare the preceding paper, *l. c.*) show a remarkable agreement in the number of their chromosomes. Thus, if we count each bivalent chromatin element of the first maturation mitosis as two, there would be the following number of univalent elements (counting in also chromatin nucleoli) in this mitosis of the following species: *Lygus pratensis*, 34; *Leptopterna dolobrata*, 34; *Calocoris rapidus*, 33; *Pœcilocapsus lineatus*, 35?; *P. goniphorus*, 34 or 36. There is not found in the *Capsidæ* such a disparity in the number of chromosomes as is found between the species of some other families (*e.g.*, the *Lygæidæ* and *Coreidæ*), so that the *Capsidæ* would appear to be a more homogeneous group. Then if the number of the chromosomes may be loosely taken as a criterion of the degree of specialization, a smaller number of chromosomes marking a more specialized stage (and this I hold to be true within certain bounds), the *Capsidæ*, like the *Reduviidæ* and *Phymatidæ*, may be considered relatively primitive *Hemiptera heteroptera*, in comparison with the *Pentatomidæ*, *Lygæidæ* and *Coreidæ*. This, it seems to me, is a vital interest in the study of the chromosomes—to find criteria for testing relationships.

5. Nabis annulatus Reut.

I had only a single testis for examination, and it showed no spermatogonic mitoses.

There is no complete rest stage in the growth period of this species (in which regard it is like certain of the *Coreidæ*). In the late telophase there is found in the nucleus (Pl. X, fig. 11) a large, usually centrally placed chromatin nucleolus (*N. 2*), with

more or less uneven contours; and attached to it is a smaller true nucleolus (*N.*) which disappears in the following prophase by gradual decrease in volume. Sometimes there are two chromatin nucleoli, generally of different volumes; since in such cases neither of these has the volume of the single one, they probably represent separated parts of the latter.

In the prophases of the first maturation mitosis, which follow immediately upon the stage just described, the chromatin nucleolus shows itself to be composed of four dumbbell-shaped (hence bivalent) parts of unequal volumes, arranged close together (*N. 2*, fig. 13). Necessarily all four parts must have been present in the preceding stage, but have been optically inseparable; the apparently single chromatin nucleolus of the growth period is made up in reality of four bivalent ones. There is great diversity in the mode of mutual apposition of the latter in the prophases; sometimes the long axes of all may be parallel, but more frequently they cross one another at varying angles; no case was seen where all four lie in the same plane. Quite frequently there are only three chromatin nucleoli in mutual contact near the centre of the nucleus, while the fourth is separated from them and placed against the nuclear membrane (fig. 12). All four are true chromatin nucleoli, maintaining throughout the growth period their dense structure, even contours, and red stain with the saffranine-violet method of Hermann, while the chromatin of the chromosomes proper stain violet. In the prophase we are considering are found also six bivalent chromosomes (portions of all of which are seen in fig. 13); these are tetrads with very wide longitudinal splitting of the type characteristic for *Anasa* (Coreid). Toward the close of the prophase these chromosomes shorten and become much more compact structures.

Pole views of the monaster stage of the first maturation mitosis (fig. 14) show in every case ten chromatin elements of comparatively large size. Four of these must correspond to the four chromatin nucleoli, and six to the six chromosomes proper of the preceding stages, since there has been no loss nor multiplication of any of these elements. Of the ten elements of the stage of fig. 14, one (*p.*) on pole view always appears round, on lateral view (*p.*, fig. 16) it shows a simple dumbbell shape; this one, much smaller than any of the others, probably represents one of

the chromatin nucleoli. The nine remaining elements are likewise all dumbbell-shaped, but on pole view of the spindle (fig. 14) each of them appears elongate, sometimes showing a split in the long axis. On lateral views of the spindle (figs. 15, 16) they sometimes appear bipartite, sometimes quadripartite. As a study of them in the preceding prophases demonstrates, each becomes placed in the equatorial plane of the spindle so that the transverse split (the line of junction of the two component univalent chromosomes) lies in the equator of the spindle, and the longitudinal split of each univalent chromosome lies perpendicular to this plane. Thus the nine larger chromatin elements of fig. 14 appear elongate on pole view of the spindle, because each of these bivalent chromosomes is composed of two univalent chromosomes with their long axes parallel to one another and to the equatorial plane of the spindle. Hence on pole view of this spindle we see a plate of (univalent) chromosomes, each seen longitudinally ; whereas such a view in the other *Hemiptera* studied by me shows the chromosomes seen from their ends, since in other *Hemiptera* it is the general rule that two univalent chromosomes are joined end to end, and not (as in *Nabis*) side to side.

All ten elements divide transversely in this mitosis, so that whole univalent elements become separated from one another. A pole view of one of the plates of daughter chromosomes resulting from this division (fig. 17) shows one smallest element (*p.*), the half of the corresponding element of figs. 14 and 16, and nine larger elements, the halves of those of fig. 14. In fig. 17 each of the nine larger univalent elements shows a well-marked longitudinal split, which had been usually hidden in the preceding monaster stage (fig. 14); it is a general rule in the *Hemiptera* that the longitudinal split becomes temporarily hidden in the monaster stage of the first maturation mitosis.

One point needs to be emphasized: there are in the prophases four chromatin nucleoli and six chromosomes, and in the monaster stage of the first mitosis again ten elements, that is, obviously the same as those in the prophases. But the four chromatin nucleoli of the prophases (figs. 12, 13) are smaller than any of the chromatin elements of the monaster stage (fig. 14), except the small element in the latter marked *p*. Accordingly three at least of the chromatin nucleoli must have increased in volume

before the latter stage. This is remarkable, since in all other *Hemiptera* studied by me the chromatin nucleoli regularly decrease somewhat in volume, generally to considerable degree, before they take their position in the equator of the spindle.

In *Coriscus ferus* Linn., the only other species of the *Nabidæ* studied, I found (preceding paper, *l.c.*) in the monaster stage of the first maturation mitosis nine bivalent chromosomes and one bivalent chromatin nucleolus. In the growth period preceding there is present in the nucleus one bivalent chromatin nucleolus of large size and a smaller one; but not a group of four bivalent chromatin nucleoli as in *Nabis*. If the chromatin nucleoli be regarded as disappearing chromosomes, for which view I have given reasons, then we may conclude that *Nobis annulatus*, by virtue of showing four of the chromatin elements on the way to disappearance, has advanced beyond the stage of *Coriscus ferus*.

6. **Corizus alternatus** Say.

Five testes of this species were studied.

Only two clear cases of spermatogonic monasters were found where all the chromatin elements could be readily counted; each of these showed fourteen elements. As Pl. X, fig. 18 shows, two of the elements are rounded and much smaller than the others (*N. 2*), and these are chromatin nucleoli. Of the twelve elongate chromosomes proper, two (those marked *A*, fig. 18) are considerably larger than the others; and one of these appears always to have the form of a rod, while the other has a bent V-shape. All fourteen elements are halved in the metakinesis.

In the early portion of the growth period each spermatocytic nucleus contains a clearly bipartite chromatin nucleolus, representing a union of the two chromatin nucleoli derived from the spermatogonia; each of its univalent components appears occasionally longitudinally split, which is unusual in the *Hemiptera*.[2] In the rest stage following (there is a complete rest stage in this species) the nucleus (fig. 19) contains a bivalent chromatin nucleolus (*N. 2*), which has increased in volume and generally is ovoid in outline; but sometimes during the whole growth period the two

[2] Dr. F. C. Paulmier, who has worked out the spermatogenesis of *Anasa tristis* De G., has demonstrated to me a longitudinal splitting of the chromatin nucleolus in the growth period of the Lygæid, *Myodocha serripes* Oliv.

univalent chromatin nucleoli may remain entirely disconnected. In this rest stage the nucleus contains also one or two larger, irregularly shaped true nucleoli (fig. 19, *N.*) which are not apposed to the chromatin nucleolus.

Pole views of the monaster stage of the first maturation mitosis (Pl. X, fig. 20) show seven chromatin elements; and lateral views of such cases show that all seven are dumbbell-shaped, and hence bivalent. The smallest of these elements is the chromatin nucleolus (*N. 2*, figs. 20, 21), and, as is generally the case in *Hemiptera*, this divides in metakinesis before the chromosomes do. Of the six chromosomes proper, one is always much larger than the others (figs. 20, 21), and this one evidently represents the union of the two largest univalent chromosomes of the spermatogonia (in fig. 21 is shown, besides the chromatin nucleolus, *N. 2*, thi largest chromosome and three of the five smaller chromosomes); and one chromosome is much smaller than the others, often little larger than the chromatin nucleolus (this is the one lying nearest to the largest chromosome in fig. 21). All these elements are transversely divided in the metakinesis. Occasionally pole views of the monaster stage of the first maturation mitosis show eight chromatin elements instead of seven; this is due to one of the seven bivalent elements having precociously divided into its univalent components. In the second spermatocyte are regularly found seven univalent elements.

Corizus annulatus in its spermatogenesis thus shows a very close similarity to *C. lateralis* Say, previously described by me.

7. **Harmostes reflexulus** Say.

The individuals collected at Woods Holl were marked by Dr. Uhler, "*Harmostes reflexulus* Say, variety"; whether a geographical race was thereby intended I cannot say.

Five testes were examined. The whole process of spermatogenesis seems exactly similar to that described by me previously for individuals of this species from Pennsylvania.

This is one of the *Hemiptera* with an *uneven* normal number of chromosomes, there being found in the spermatogonia thirteen chromatin elements, namely, two smaller chromatin nucleoli (*N. 2* of figs. 22 and 23) and eleven larger chromosomes proper. The uneven normal number of chromosomes being a relatively rare phenomenon, it having been observed so far only in four species of

Hemiptera (described in my previous paper, *l. c.*), I have counted in the testes of the Woods Holl individuals the chromatin elements in all the cases of spermatogonic monasters which were favorable for such counting, with the following results: Nine spermatogonia showed exactly thirteen elements; in one case I could not determine whether thirteen or fourteen were present. These cases from four different testes, as well as those from four testes of Pennsylvania individuals previously described by me, are sufficient to show that the uneven number is not an individual variation, due *e.g.* to some pathological condition, but is probably characteristic of every individual of the species.

The uneven normal number of chromosomes which I have demonstrated also for *Protenor belfragei*, *Alydus eurinus* and *Œdancala dorsalis*, represents a stage in the change of the number of chromosomes from one even number to the next successive even number. For *Protenor* I have shown that the uneven spermatogonic number is produced by a failure of two of the spermatogonic chromosomes to separate from one another. This I can now prove for *Harmostes* also. For while in most of the monaster stages, as in fig. 22, all eleven chromosomes appear more or less simply rod-shaped, in a few cases, as in fig. 23, one of the eleven shows a well-marked transverse constriction. Were this constriction a complete division, there would be the even number twelve. Hence, for *Harmostes* the ancestral number of chromosomes must have been twelve, and if, as is the case in *Protenor*, the odd bivalent chromosome is destined to change from a chromosome into a chromatin nucleolus, in the course of time ten chromosomes will be the number for the species.

In conclusion, I would again call attention to the importance of studying the chromosomal relations comparatively in a large number of species of a group. By such investigations not only may much of importance be obtained regarding the evolution of cell structures themselves, but by implication a criterion may thereby be obtained for testing genetic relationships. In opening up this line of research, I have drawn attention so far mainly to the numerical relations of the chromosomes, and to the chromatin nucleoli as representing chromosomes on the way to disappearance during progressive evolution. These are the facts most easily

determined; and there is the surety in such study that the chromosomes are relatively large structures, which exist in fact and are not artificially produced by the mode of preparation necessary for their study. The chromosomes are not apparently formed *de novo*—at least there is as yet no good proof in any case that they are so formed; while, on the contrary, there is a considerable amount of evidence to show that they are structures which persist from generation to generation, even though this is a persistence nvolving a great amount of metabolic change. Astral radiations appear and disappear, or at least disappear as *radiations;* nucleoli are apparently accumulations of metabolic substances of no morphological regularity, as I have shown in another place;[3] and recent experimental studies would show, though perhaps in contradiction to the anatomical studies, that the centrosomes may be formed anew. But the chromosomes show more fully than any of these cellular structures a certain degree of morphological stability, and this fact, taken in connection with their greater adaptability for study, entitles them to a basic place in the study of the cell's evolution, as well as in the study of evolution in general.

EXPLANATION OF PLATE X.

All figures have been drawn to the same scale with the camera lucida at the level of the base of the microscope, with the Zeiss homogeneous immersion $\frac{1}{12}$, ocular 4, tube length 180 mm.

The bounding line in figs. 1–4, 6–10, 14–18, and 20–23 represents the cell membrane; in figs. 5, 11–13, and 19, the nuclear membrane. In lateral views of the mitotic spindles (figs. 2, 4, 7, 15, 16, 21) the mantle fibres are the only achromatic elements shown, and are represented thicker than they are in reality.

PLATE X, fig. 1.—*Tingis clavata*, pole view of monaster stage of the first maturation mitosis.

Fig. 2.—*Idem*, lateral view of the same stage.

Fig. 3.—*Corixa verticalis*, pole view of monaster stage of the first maturation mitosis.

Fig. 4.—*Idem*, lateral view of the same stage.

Fig. 5.—*Idem*, nucleus in post-synapsis stage.

Fig. 6.—*Cymus luridus*, pole view of monaster stage of the first maturation mitosis.

Fig. 7.—*Idem*, lateral view of the same stage.

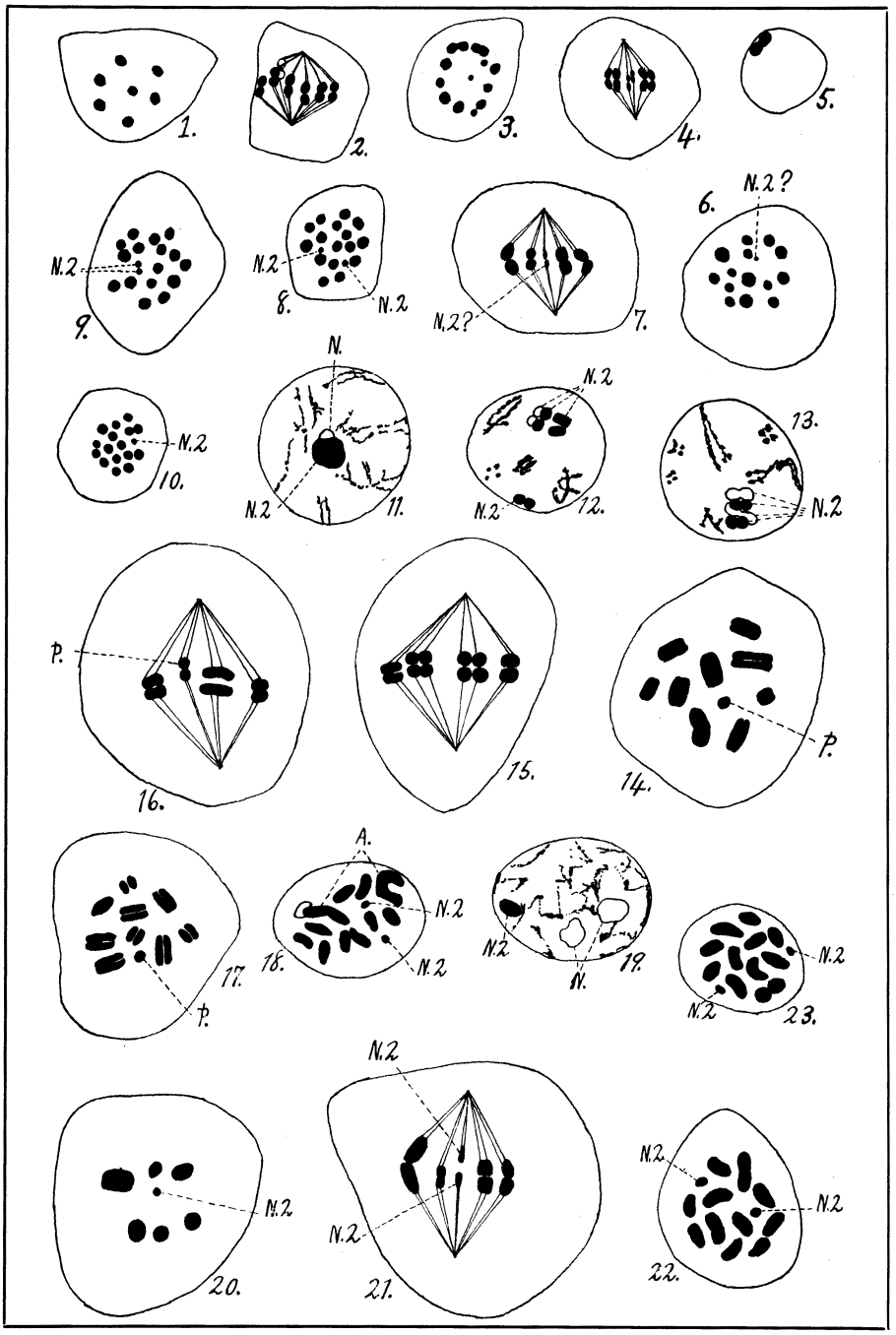
Figs. 8, 9.—*Lygus pratensis* var., pole views of monaster stage of first maturation mitosis.

Fig. 10.—*Idem*, pole view of monaster stage of second maturation mitosis.

Fig. 11.—*Nabis annulatus*, nucleus in growth period (late telaphase)

[3] *Journal of Morphology*, Vol. XV, 1898.

Figs. 12, 13.—*Idem*, nuclei in prophases of first maturation mitosis.
Fig. 14.—*Idem*, pole view of monaster stage of first maturation mitosis.
Figs. 15, 16.—*Idem*, lateral views of metakinesis of first maturation mitosis.
Fig. 17.—*Idem*, pole view of one plate of daughter chromosomes, early anaphase of first maturation mitosis.
Fig. 18.—*Corizus alternatus*, pole view of monaster stage of a spermatogonium.
Fig. 19.—*Idem*, nucleus of first spermatocyte, rest stage.
Fig. 20.—*Idem*, pole view of monaster stage of first maturation mitosis.
Fig. 21.—*Idem*, lateral view of the same stage.
Figs. 22, 23.—*Harmostes reflexulus* var., pole views of monaster stage of spermatogonia.



MONTGOMERY. CHROMOSOMES OF HEMIPTERA HETEROPTERA.